

Hepatocytes from highly repopulated primary (#531) and tertiary (#631) mice were hybridized with either human or mouse total genomic DNA. The percentage of cells positive for the human probe or murine probe were scored. Controls were pure human and mouse hepatocytes or an equal mix of human and mouse hepatocytes.

If the human cells found in chimeric livers were the product of cell fusion, many hepatocytes would be expected to be double-positive for both probes and hence the percentages of cells positive for mouse and human DNA would exceed 100%. Instead, the sum of percentages closely approximated 100% as it did in the mix of human and murine hepatocytes. Thus, double-positive cells (fusion products) could not account for the majority of human cells.

Table

	Mouse probe positive (%)	Human probe positive (%)	Sum of percentages
Murine hepatocytes	87/87 (100)	0/103 (0)	100
Human hepatocytes	0/99 (0)	107/107 (100)	100
Mix	38/101 (38)	68/115 (59)	97
Chimeric mouse #531	15/100 (15)	95/111 (86)	101
Chimeric mouse #631	23/87 (26)	69/94 (73)	99

Supplementary Figure 1

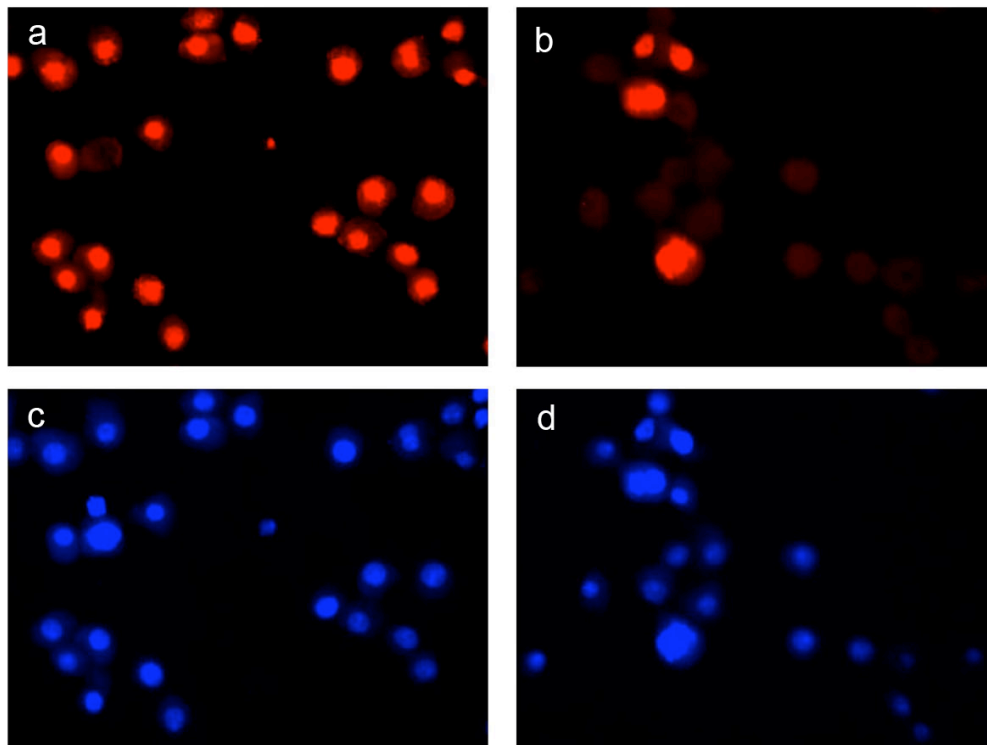


Figure S1 legend

Hepatocytes from mouse #531 (a-d). FISH with human (a) and mouse (b) probe is shown. The corresponding Hoechst stain is shown beneath each FISH panel.

## **MATERIALS & METHODS**

### **Total genomic DNA probes**

Probes were generated by nick translation of total mouse and human genomic DNA. Cy3-dUTP incorporation was carried out according to manufacturer's recommendations (Invitrogen). Final probe concentration was 200 ng/1.

### **Fluorescence *in situ* hybridization**

Slides with attached cells were treated with RNase at 100 mg/ml for 1 h at 37°C and washed in 2X SSC for three 3-min rinses. Following wash steps, slides were dehydrated in 70, 90 and 100% ethanol at 3 min each. Chromosomes were denatured at 75°C for 3 min in 70% formamide/2X SSC, followed by dehydration in ice cold 70, 90 and 100% ethanol for 3 min each. Probe cocktails were denatured at 75°C for 10 min and pre-hybridized at 37°C for 30 min. Probes were applied to slides and incubated overnight at 37 °C in a humid chamber. Post-hybridization washes consisted of three 3-min rinses in 50% formamide/2X SSC and three 3-min rinses in PN buffer (0.1 M Na<sub>2</sub>HPO<sub>4</sub> • 0.1 M NaH<sub>2</sub>PO<sub>4</sub>, pH 8.0, •2.5% Nonidet NP-40), all at 45°C. Slides were then counterstained with Hoechst (0.2ug/ml), cover-slipped and viewed under UV fluorescence (Zeiss).

## Supplementary Fig.2

Cultured hepatocytes from three mice were analyzed. M790 had 10%, M697 30% and M785 60% human repopulation. (a) Basal expression of liver-specific genes in the 3 samples, normalized to mouse actin mRNA. (b-h) Induction of mRNAs involved in drug metabolism in response to beta-naphthoflavone (BNF), phenobarbital (PB) and rifampicin (Rif). Std. = in non-induced cultures. (b) *CYP3A4*, (c) *CYP2B6*, (d) *CAR* (nuclear hormone receptor), (e) *MDR1* (transporter), (f) *MRP*, (g) *BSEP*(transporter), (h) *PXR* (nuclear hormone receptor). The induction of *CYP3A4* by phenobarbital was even more striking than at the enzyme level.

